Gene Editing of Human Embryonic Stem Cells via an Engineered Baculoviral Vector Carrying Zinc-finger Nucleases.

Journal: Mol Ther

Publication Year: 2011

Authors: Y Lei, C L Lee, K I Joo, J Zarzar, Y Liu, B Dai, V Fox, P Wang

PubMed link: 21326219

Funding Grants: Development of Baculoviral Vectors for Gene Editing of Human Stem Cells, USC Center for Stem Cell and Regenerative Medicine: Shared Research Laboratory and Course in Current Protocols in Human Embryonic Stem Cell Research

Public Summary:
Stem cells potentially provide powerful tools for analyzing the function of specific genes, an experimental technology that has proven revolutionary in the mouse. However, it has been difficult to make targeted changes in the genome of human embryonic stem cells. We developed a new technique to produce specific genetic modifications in human embryonic stem cells. This technology will be useful in developing new stem cell lines to study gene function and the role of genes in diseases.

Scientific Abstract:
Human embryonic stem (hES) cells are renewable cell sources that have potential applications in regenerative medicine. The development of technologies to produce permanent and site-specific genome modifications is in demand to achieve future medical implementation of hES cells. We report herein that a baculoviral vector (BV) system carrying zinc-finger nucleases (ZFNs) can successfully modify the hES cell genome. BV-mediated transient expression of ZFNs specifically disrupted the CCR5 locus in transduced cells and the modified cells exhibited resistance to HIV-1 transduction. To convert the BV to a gene targeting vector, a DNA donor template and ZFNs were incorporated into the vector. These hybrid vectors yielded permanent site-specific gene addition in both immortalized human cell lines (10%) and hES cells (5%). Modified hES cells were both karyotypically normal and pluripotent. These results suggest that this baculoviral delivery system can be engineered for site-specific genetic manipulation in hES cells.